WE CLAIM:

1. A method for identifying cytotoxic mutant proteins capable of binding to a target cell, comprising:

(A) selecting a heteromeric protein toxin having a toxic subunit and a binding subunit;

(B) generating a library of microorganism clones producing variant protein toxins of said heteromeric protein toxin by incorporating mutations into the binding subunit DNA of the heteromeric protein toxin; and

(C) screening the variant protein toxins of said library against said target cell by isolating clones or pools of clones producing said variant protein toxins, treating preparations of said target cell with said variant protein toxins, and selecting a cytotoxic mutant protein or pool of cytotoxic mutant proteins that inhibits or kills said target cell.

2. The method as claimed in claim 1, wherein said target cell is eukaryotic.

3. The method as claimed in claim 1, wherein said library comprises bacteria or bacterial supernatants containing said variant protein toxins.

4. The method as claimed in claim 1, wherein said library comprises yeast or yeast supernatants containing said variant protein toxins.

5. The method as claimed in claim 1, wherein said binding subunit DNA is in a plasmid in said microorganism.

6. The method as claimed in claim 1, wherein said mutation is incorporated into said binding subunit by use of a combinatorial cassette method comprising:

(A) preparing synthetic mutant oligonucleotides capable of annealing with a corresponding wild type oligonucleotide from said binding subunit;

(B) annealing said synthetic oligonucleotide from said binding subunit to an

overlapping wild type oligonucleotide to form a double stranded sequence;

(C) creating a combinatorial cassette by mutually primed synthesis of said oligo sequence; and,

(D) incorporating said cassette into a vector containing a gene for said toxin.

7. The method as claimed in claim 1 wherein said mutation is incorporated into said binding subunit by means of a unique site elimination method.

8. The method as claimed in claim 1 wherein said heteromeric protein toxin is selected from a group comprising prokaryotic or eukaryotic proteins or protein fusion constructs capable of blocking protein synthesis.

9. The method as claimed in claim 1 wherein said heteromeric protein toxin is selected from a group comprising Shiga toxin, Shiga-like toxins, ricin, abrin, gelonin, crotin, pokeweed antiviral protein, saporin, momordin, modeccin, sarcin, diphtheria toxin and *Pseudomonas aeruginosa* exotoxin A.

10. The method as claimed in claim 9 wherein said heteromeric protein toxin is Shiga toxin or Shiga-like toxin 1.

11. The method as claimed in claim 10 wherein said random mutation is incorporated into loop regions at residues 15-19, 30-33 or 58-64.

12. The method as claimed in claim 10 wherein said random mutation is incorporated into loop regions at residues 15-19 or 30-33.

13. The method as claimed in claim 2 wherein said target cell is a tumour cell.

14. The method as claimed in claim 13 wherein said target cell is a breast cancer cell.

15. The method as claimed in claim 14 wherein said breast cancer cell is SK-BR-3 or CAMA-I.

16. The method as claimed in claim 1 wherein said binding subunit is derived from the B-subunit template of either Shiga toxin or related Shiga-like toxins, or homologous counterparts from *E. coli* heat labile enterotoxins, cholera toxin, pertussis toxin or the receptor binding domain of ricin.

17. A method of killing or inhibiting a target cell comprising treating said target cell with said cytotoxic mutant protein or pool of proteins of claim 1.

18. A method for identifying therapeutic proteins having binding specificity for a target cell comprising:

(A) identifying cytotoxic mutant proteins by the method as claimed in claim 1; and,

(B) screening said cytotoxic mutant proteins against non-target cells by treating preparations of non-target cells with said cytotoxic mutant proteins, and selecting a therapeutic protein or pool of therapeutic proteins that are less effective at inhibiting or killing said non-target cells than at inhibiting or killing said target cells.

19. A method for constructing diagnostic probes for detecting the presence of a cell surface marker comprising:

(A) selecting by the method as claimed in claim 1 the cytotoxic mutant protein;

(B) selecting from said library of microorganism clones a clone which produces said cytotoxic mutant protein;

(C) preparing a diagnostic DNA sequence by incorporating a marker DNA encoding for a detectable marker into a binding subunit DNA sequence in the selected clone; and,

(D) generating diagnostic probes from said diagnostic DNA sequence.

20. A method for constructing diagnostic probes as claimed in claim 19 wherein said marker DNA codes for green-fluorescent protein (GFP).

21. A method for constructing diagnostic probes for detecting the presence of a cell surface marker comprising:

(A) identifying by the method as claimed in claim 1 a cytotoxic mutant protein or pool of proteins for use as a diagnostic probe;

(B) optionally modifying said cytotoxic mutant protein or pool of proteins by dissociation or by inactivation of said toxic subunit; and,

(C) optionally labelling said cytotoxic mutant protein or pool of proteins with a detectable marker.

22. A method for constructing a medicament having binding specificity comprising:

(A) selecting, by the method as claimed in claim 18, a therapeutic protein having binding specificity;

(B) optionally modifying said therapeutic protein by dissociation or by inactivation of said toxic subunit; and,

(C) binding a medicine to said binding subunit to form a medicament.

23. A method for constructing a medicament having binding specificity comprising:

(A) selecting by the method as claimed in claim 1 the cytotoxic mutant protein;

(B) selecting from said library of microorganism clones a clone which produces said cytotoxic mutant protein;

(C) preparing a medicament DNA sequence by incorporating a medicinal DNA encoding for a medicinal polypeptide into a binding subunit DNA sequence in the selected clone; and,

(D) generating a medicament from said medicament DNA.

24. A method for treating a condition requiring targeting a medicine to a target

cell occurring in a host organism comprising selecting a medicamentby the method as claimed in claim 22 and administering to said host organism an effective amount of said medicament.

25. A method for treating a condition requiring targeting a medicine to a target cell occurring in a host organism comprising selecting a medicament by the method as claimed in claim 23, and administering to said host organism an effective amount of said medicament.

26. A kit useful for performing the method of claim 1, said kit comprising said heteromeric protein toxin and suitable supports useful in performing said method.

1
KEFTLDFSTAKTYVDSLNVIRSAIGTPLQTISSGGTSLLMIDSGSGDNLFAV

DVRGIDPEEGRFNNLRLIVERNNLYVTGFVNRTNNVFYRFADFSHVTFPGT

TAVTLSGDSSYTTLQRVAGISRTGMQINRHSLTTSYLDLMSHSGTSLTQS

VARAMLRFVTVTAEALRFRQIQRGFRTTLDDLSGRSYVMTAEDVDLTLN

WGRLSSVLPDYHGQDSVRVGRISFGSINAILGSVALILNCHHHASRVARM

293
ASDEFPSMCPADGRVRGITHNKILWDSSTLGAILMRRTISS

FIG. 1A

1
15 19 30 33
TPDCVTGKVEYTK[YNDDDT]FTVKVGDKEL[FTNR]WNLQSLLLSAQITGMTV
58 64 69
TIKTNAC[HNGGGFS]EVIFR

FIG. 1B